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MOLECULAR MECHANISM OF RADIATION RESISTANCE AND RECOVERY OF BAC--ETC(U)
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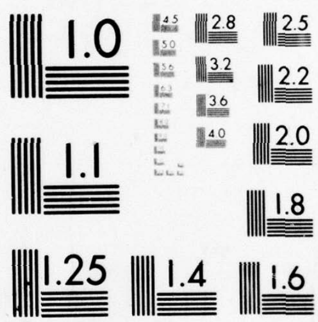

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18. SUPPLEMENTARY NOTES

The findings in this report are not to be construed as an official Department of the Army position, unless so designated by other authorized documents.

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Direct rejoining of DNA (by ligase) occurs in dormant spores in a radiation resistant strain but not in a radiation sensitive strain. The ability to rejoin DNA seems to be responsible for the extensive shoulder in the radiation survival curves, as well as for salt tolerance of a particular strain. Calcium form spores have increased resistance as well as increased water binding capacity. This seems to be due to the ability of calcium to convert weak cationic exchange groups in the spore such as $R\text{-}COO^{(-)}$ into strong anionic exchange groups such as $R\text{-}COO^{(-)}Ca^{++}$ + $5H_2O$.

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20. ABSTRACT CONTINUED

Freeze-thawing causes DNA breakage in E. coli; this seems to be the mechanism of cell death if breaks are not repaired. Mild heat triggers DNA breakage in E. coli; the enzyme apurinic acid endonuclease in the E. coli cell is implicated in producing the breaks. These breaks may or may not be reversed by DNA ligase, thus resulting in survival or death of the cells, respectively.

FINAL TECHNICAL REPORT

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See separate page attached.

8. SCIENTIFIC PERSONNEL SUPPORTED BY THIS PROJECT AND DEGREES AWARDED DURING THIS REPORTING PERIOD:

Dr. T. W. Kang-received Ph.D. degree May, 1976.

Dr. E. DeMet-received a Ph.D. degree May, 1976.

Miss S. Bhatarakamol-received M.S. degree December, 1976.

Mr. Melvin D. Long-Ph.D. candidate-plans to graduate May, 1978

Mr. James P. Grice-Ph.D. candidate since Sept. 1976.

Alexandra Chicz-Ph.D. candidate.

Teri L. Hammer, senior undergraduate worked in our lab during Summer and Fall, 1977.

12880-L

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1. Highlights of Research Findings

a. Direct rejoining of DNA (by ligase) occurs in dormant spores in a radiation resistant strain but not in a radiation sensitive strain. The ability to rejoin DNA seems to be responsible for the extensive shoulder in the radiation survival curves, as well as for salt tolerance of a particular strain.

b. Calcium form spores have increased resistance as well as increased water binding capacity. This seems to be due to the ability of calcium to convert weak cationic exchange groups in the spore such as $R\text{-}COO^-$ into strong anionic exchange groups such as $R\text{-}COO^-Ca^{++} + 5H_2O$.

c. Freeze-thawing causes DNA breakage in E. coli; this seems to be the mechanism of cell death if breaks are not repaired.

d. Mild heat triggers DNA breakage in E. coli; the enzyme apurinic acid endonuclease in the E. coli cell is implicated in producing the breaks. These breaks may or may not be reversed by DNA ligase, thus resulting in survival or death of the cells, respectively.

2. Discussion of Research Findings

Our research effort during the reporting period was concerned with the molecular mechanisms by which DNA strands are broken and repaired, and how these mechanisms are related to resistance, recovery or death of injured cells, including bacterial spores and vegetative cells.

a. Role of DNA-ligase in spore resistance.

Radiation injury and repair of spore DNA was considered in relation to spore cytoplasmic enzymes, particularly DNA-ligase. In addition to stabilization by typical protective spore structures, spore survival also seems to depend on an efficient system of rejoining of initial DNA breaks. Radiation resistant spores may rejoin at least 50% of the initial DNA breaks, while radiation sensitive spores show no detectable DNA rejoining (ligase) activity (Durban, Grecz & Farkas, 1974). The operation of this enzymatic DNA maintenance system in apparently dormant non-germinated spores may be of great biological survival value and has not been suspected to exist until its discovery in our laboratory in 1974.

The experiments show that the DNA-breakage-DNA rejoining capacity operative in dormant bacterial spores is responsible for the shoulder portion of radiation survival curves. Furthermore, the size of the shoulder is significantly related to the overall radiation resistance; this is in contrast to the relative unimportance of the slopes.
Failure to rejoin DNA breaks in the dormant spore may eventually result in spore death.

Differences in radiation resistance of 14 strains of Clostridium botulinum spores could be correlated by computer analysis with differences in the lag or shoulder portion (L) of their respective radiation survival curves. The exponential decline portion (E) of the survival curves were nearly identical for all

14 strains. Autoradiographic, and diphenylamine assays indicated that different strains contained either one or two genomes per spore, however, no relationship could be detected between number of genomes and radiation resistance of the spores. Alkaline sucrose gradient sedimentation of ^{3}H-DNA indicated that L was characterized by production of DNA single-strand breaks (SSB). Radiation resistant strain 33A rejoined 50-90% of the initial SSB during irradiation or shortly thereafter, i.e. while the spores were still in the cryptobiotic dormant state. Rejoining of SSB seems to be due to high DNA-ligase activity in strain 33A. On the other hand, the radiation sensitive strain 51B showed no shoulder (L) and very little or no SSB rejoining. The exponential decline portion (E) seems to be associated with those lesions which cannot be repaired during irradiation or germination. It is thought that repair of these lesions is attempted after germination and initiation of metabolism and may involve DNA excision-resynthesis, and recombination, especially in those strains containing two genomes per spore. These repair mechanisms are error-prone and thus frequently result in cell death characteristic of the E portion of the survival curve. (Grecz, Lo, Kang & Farkas, 1977; Grecz, Wiatr, et.al., 1978).

The relationship between radiation resistance and salt sensitivity of spores of Clostridium botulinum and the possible implications with respect to DNA breaking-and-rejoining in irradiated spores is described in a joint manuscript with Dr. Farkas of the Central Food Research Institue Budapest, Hungary and Dr. T.A. Roberts of the British Meat Research Institute, Bristol, U.K. (accepted by Appl. and Environm. Microbiol).

Experiments are in progress by J. Grice within his Ph.D. program to characterize the DNA-rejoining system of spores of the highly radiation resistant C. botulinum 33A. The strategy involves the study of specific cofactors and inhibitors of DNA-ligase with respect to their effect on DNA breakage and DNA repair as the result of gamma irradiation.

Our finding of DNA rejoining in dormant spores apparently involving enzyme activity (DNA ligase) is unexpected, and untill further confirmation should be viewed with all due caution. This finding is contrary to the established view that in dormant bacterial spores all enzymatic processes are arrested by the practically dehydrated state in the spore cytoplasm. Furthermore, dormant spores contain low levels of nucleotides (P. Setlow) and therefore the NAD (or ATP) necessary for DNA rejoining may not be available. On the other hand, contrary to these objections in vivo and in vitro results so far strongly indicate that enzymatic DNA rejoining does in fact occur (at least in radiation resistant strains) while the spores are still in the dormant state.

b. The molecular mechanism of the stable "dehydrated" nature of spores.

These studies continue our long standing bioinorganic approach to the mechanism of spore resistance and dormancy. From this point of view the coordination chemistry of Ca (II) DPA and other ligands was recently extended to the study of complexing of bound calcium with water molecules (see papers with Rajan & Tang 1968-1977). Knowledge of the physico-chemical environment in the spore is essential for explaining the activity (or inactivity) of DNA repair enzymes investigated in this project. Our recent TGA, DTA and DSC experiments indicate that spores rich in calcium have higher water binding affinity and at the same time have a higher resistance to heat. We attribute this to the ability of calcium to convert weak cation exchange groups in the spore, such as $R\text{-}COO^-$ into strong anion exchange groups such as $R\text{-}COO^-Ca^{++} + 5H_2O$.

Anion exchangers of this type would strongly bind and immobalize relatively large quantities of water thus creating an apparently "dehydrated" environment in the spores. This model may explain some of the observations to date on calcium-form spores particularly with respect to their special properties of resistance and dormancy as related to calcium binding (see manuscript for Biophys. J.; Grecz, Gal & Sztatisz, 1978).

c. The nature of initial events of DNA injury by biophysical stress: *E. coli*.

Since investigation of spores is greatly complicated by their tough and multiple protective cytology, we find it expedient to use well defined and readily available genetic mutants of *E. coli* for investigation of relevant initial events of DNA injury by various biophysical stresses. This combined approach enhances progress in the elucidation of the biophysical mechanisms of cell resistance and injury. In this line, we have discovered that not only radiation but also heat and freezing are capable of inducing DNA breakage and that such breakage may result in cell death (Alur & Grecz, 1975). Furthermore, we have discovered that DNA breakage induced by mild heat (ca 50°C) is enzymatic in nature. Genetic experiments implicate the cell's own endonuclease in this type of DNA breakage, particularly the apurinic acid endonuclease (Grecz & Bhatarakamol, 1977). The important recognition concerning the cell's endonuclease is that mild heating (50-60°C) is able to trigger endonucleolytic DNA breakage which seems to be the initial event in injury by mild heat.

3. Progress Report: Publications

Durban, E., E. Durban and N. Grecz. 1974. Production of spore spheroplasts of *Clostridium botulinum* and DNA extraction for density gradient centrifugation. Can. J. Microbiol. 20: 353-358.

Durban, E., Grecz, N. and J. Farkas. 1974. Direct enzymatic repair of DNA single strand breaks in dormant spores. J. Bacteriol. 118: 129-138.

Alur, M.D., and N. Grecz. 1975. Mechanisms of injury of *E. coli* by freezing and thawing. Biochem. Biophys. Res. Commun. 62, 308-312.

Kang, T.W. and N. Grecz. 1975. Early events in germinating spores of *Clostridium botulinum* 33A and 62A: Chromosome segregation and cell growth patterns. Spores. VI pp. 513-519.

Grecz, N., H. Lo, T.W. Kang and J. Farkas. 1977. Characteristics of radiation survival curves of spores of *Clostridium botulinum* strains. pp. 603-630. In A.N. Barker, J. Wolf, D.J. Ellar, D.J. Dring and G.W. Gould (eds). SPORES 1976, Vol. 2. Academic Press, London. Released in Oct. 1977.

Rajan, K.S. and N. Grecz. 1977. Chelation characteristics of calcium in relation to water binding and heat resistance of bacterial endospores. pp. 527-543. In A.N. Barker, J. Wolf, D.J. Ellar, D.J. Dring and G.W. Gould (eds). SPORES 1976, Vol. 2. Academic Press, London. Released in Oct. 1977.

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Alur, M.D., N.F. Lewis and N. Grecz. 1977. Evidence of chemical protection against freeze-induced DNA breakage. FEMS-Letters, 1: 367-369.

Grecz, N. and T.L. Hammer. 1977. Patterns of cell arrangement and nuclear segregation in radiation damaged Micrococcus luteus. FEMS-Letters, 2: 145-147.

Grecz, N. and S. Bhatarakamol. 1977. Apurinic acid endonuclease implicated in DNA breakage in Escherichia coli subjected to mild heat. Biochem. Biophys. Res. Comm. 77: 1183-1188.

In print

Kiss, I., C.O. Rhee, N. Grecz, T.A. Roberts. 1978. Relation between radiation resistance and salt snesitivity of spores of five strains of Clostridium botulinum types A, B and E. Appl. and Environm. Microbiol., Accepted 20 Dec 1977.

Rajan, K.S., R. Jaw and N. Grecz. 1978. The role of chelation and water binding by calcium in the dormancy and heat resistance of bacterial endospores. Bioinorganic Chem., Accepted Aug 15, 1977.

In progress

Grecz, N., S. Gal and J. Sztatisz. 1978. Thermal studies of water sorption and heat resistance of bacterials spores. Biophys. J., In final stages of revision, Jan., 1978.

Bhatarakamol, S. and N. Grecz. 1977. DNA-breakage in Escherichia coli B/r chromosome in vivo at temperatures of 37°C to 100°C. J. Bacteriol. In revision, Jan., 1978

Grecz, N., M.D. Alur and J. Farkas. 1978. Synergistic effect of UV and gamma radiation: Induction of initial lesions in E. coli DNA. J. Food Safety, Submitted July 14, 1977, Revised & resubmitted Dec. 22, 1977.

Grecz, N., G. Suchanek and T. Miura. 1978. Role of N-ethylmaleimide in thermo-restoration of hydrated bacterial spores. Presented at Intl. Congr. on Radiation Chemistry. Kesthely, Hungary, June 2-7, 1976. Manuscript submitted June 18, 1976. Accepted, waiting for proofs.

Grecz, N., C. Wiatr, E. Durban, T. Kang and J. Farkas. 1978. Bacterial spores: Biophysical aspects of recovery from radiation injury. Symposium of the First International Congress on Engineering and Food, Boston, Mass. Manuscript submitted Sept. 8, 1976. Accepted, waiting for proofs.

Grecz, N. and C. Rhee. 1976-1978. Mechanism of chromosome segregation in bacteria: Evidence of nuclear DNA synthesis independent of cell wall elongation. Science. Manuscript in preparation.

Grecz, N. and L.M. Braune. 1976-1978. Characterization of a plasmid mediating resistance to kanamycin and neomycin in C. botulinum 62A. J. Bacteriol., Manuscript in preparation.

Graduate Theses in the Microbial Biophysics Laboratory Pertinant to the Proposal.

Alur, M.D. 1975. Biophysical aspects of radiation and freezing effect. M.S. Thesis.

Braune, L.M. 1975. Isolation and characterization of the plasmid mediating resistance to Kanamycin and Neomycin. M.S. Thesis.

Kang, T.W. 1976. DNA replication, segregation, and chromosome distribution patterns during early outgrowth. Ph.D. Thesis, May, 1976.

Long, M. 1976. Injury and repair mechanisms of DNA in bacteria by freezing and thawing, Ph.D. Thesis in progress.

Bhatarakamol, S. 1976. Thermally induced DNA damage and DNA repair in *Escherichia coli* . M.S. Thesis

Grice, J. 1978. *In vitro* characterization of direct DNA rejoining mechanism in dormant radiation damaged spores of *Clostridium botulinum* 33A and 51B. Ph.D. Thesis in progress.

1976 Lectures and Conference Presentations

May 18-21. Biophysical and biochemical aspects of radiation injury and cell repair. Presented at First Research Coordination Meeting on the Wholesomeness of the Process of Food Irradiation. Convened by the International Atomic Energy Agency and the Food and Agriculture Organization of the United Nations at the National Institute of Science and Technology, Manila, The Philippines.

June 2-7. Role of N-ethylmaleimide in thermorestoration of hydrated bacterial spores. Presented at Intl. Congr. on Radiation Chemistry. Kesthely, Hungary.

June 10. Lecture sponsored by the Hungarian Society of Microbiology and Food Technology. "Mechanism of combined action of radiation and heat on bacterial spores in relation to chromosome structure and function". Delivered at the Technical University, Budapest.

August 9-13. Bacterial spores: Biophysical aspects of recovery from radiation injury. Presented at the First International Congress on Engineering and Food, Boston, Mass.

1977 Invitations:

(i) to contribute Chaper 14 "Action of Radiation on Microorganisms and Viruses" to a proposed book by CRC entitled "Preservation of Food by Ionizing Radiations" E.S. Josephson and M.S. Peterson, editors and (ii) to present a paper entitled "In-vivo Evidence for the Role of DNA-ligase in Radiation Resistance of *Clostridium botulinum* 33A" at the Intl. Symp. on Food Preservation by Irradiation, Wageningen, the Netherlands, Nov. 21-25, 1977. Could not attend, but the paper was presented by co-author, Dr. Farkas. (iii) to participate in the U.S.A.-Australia Workshop on Basic Radiation Resistance Mechanisms of Bacterial Spores held 10-13 Oct. 1977 at the University of Wisconsin, Madison.

1978 Invitations:

To participate in the Second Research Coordination Meeting on the Wholesomeness of the Process of Food Irradiation jointly sponsored by the Food and Agricultural Organization of the United Nations and the International Atomic Energy Agency and to be held at the Catholic University, Leuven, Heverlee, Belgium from 23-26 May 1978.